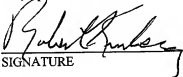


3508 Rec'd PCT/PTO 14 DEC 2001

FORM PTO-1590 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 31671-176197
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (if known, see 37 CFR 1.5) Unassigned 10/009950
INTERNATIONAL APPLICATION NO. PCT/JP00/04132	INTERNATIONAL FILING DATE June 23, 2000	PRIORITY DATE CLAIMED June 25, 1999	
TITLE OF INVENTION GOODPASTURE'S SYNDROME MODEL MOUSE			
APPLICANT(S) FOR DO/EO/US Akira NAKAMURA, Toshihiro NUKIWA and Toshiyuki TAKAI			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)). a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input checked="" type="checkbox"/> has been communicated by the International Bureau. (attach form IB 308) c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input checked="" type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). a. <input checked="" type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input checked="" type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).			
Items 11 to 20 below concern document(s) or information included:			
11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input checked="" type="checkbox"/> Other items or information: A copy of the IB-304 20a. <input type="checkbox"/> For purposes of examination, please insert the annexes to the IPER, so that the application will comprise the following pages of the English translation: Specification: Original pages Amended pages Claims: Original claims Amended claims			

U.S. APPLICATION NO. 10/009950 International Application No. PCT/JP04/013 Attorney's Docket Number 31671-176197		21. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT = \$890.00																																																																		
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)). <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 20%;">CLAIMS</th> <th style="width: 20%;">NUMBER FILED</th> <th style="width: 20%;">NUMBER EXTRA</th> <th style="width: 20%;">RATE</th> <th style="width: 20%;">\$</th> </tr> </thead> <tbody> <tr> <td>Total claims</td> <td>11 - 20 =</td> <td>0</td> <td>x \$18.00</td> <td>\$</td> </tr> <tr> <td>Independent claims</td> <td>4 - 3 =</td> <td>1</td> <td>x \$84.00</td> <td>\$84.00</td> </tr> <tr> <td colspan="3">MULTIPLE DEPENDENT CLAIMS(S) (if applicable)</td> <td>+ \$280.00</td> <td>\$</td> </tr> <tr> <td colspan="4" style="text-align: right;">TOTAL OF ABOVE CALCULATIONS =</td> <td>\$974.00</td> </tr> <tr> <td colspan="4"> <input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. </td> <td>+</td> </tr> <tr> <td colspan="4" style="text-align: right;">SUBTOTAL =</td> <td>\$974.00</td> </tr> <tr> <td colspan="4"> Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)). </td> <td>\$</td> </tr> <tr> <td colspan="4" style="text-align: right;">TOTAL NATIONAL FEE =</td> <td>\$974.00</td> </tr> <tr> <td colspan="4"> Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property </td> <td>+ \$40.00</td> </tr> <tr> <td colspan="4" style="text-align: right;">TOTAL FEES ENCLOSED =</td> <td>\$1014.00</td> </tr> <tr> <td colspan="4"></td> <td>Amount to be refunded: \$</td> </tr> <tr> <td colspan="4"></td> <td>charged: \$</td> </tr> </tbody></table>		CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	Total claims	11 - 20 =	0	x \$18.00	\$	Independent claims	4 - 3 =	1	x \$84.00	\$84.00	MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ \$280.00	\$	TOTAL OF ABOVE CALCULATIONS =				\$974.00	<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				+	SUBTOTAL =				\$974.00	Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	TOTAL NATIONAL FEE =				\$974.00	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				+ \$40.00	TOTAL FEES ENCLOSED =				\$1014.00					Amount to be refunded: \$					charged: \$	a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1014.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>22-0261</u> . A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO: VENABLE P.O. Box 34385 Washington D.C. 20043-9998 Phone No. 202-962-4800 Fax No. 202-962-8300	
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		 SIGNATURE Robert Kinberg NAME 26,924 REGISTRATION NUMBER																																																																		

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Akira NAKAMURA et al.

Appl. No. Unassigned

Filed: December 14, 2001

For: GOODPASTURE'S SYNDROME
MODEL MOUSE

Int'l. Appln. No.: PCT/JP00/04132

Int'l. Filing Date: June 23, 2000

Atty. Docket No. 31671-176197

Customer No.



26694

PATENT TRADEMARK OFFICE

Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to calculation of the fees, please amend the claims attached to the specification as follows:

5. (Amended) The method for screening a remedy for Goodpasture's syndrome according to claim 3, wherein a comparative evaluation with a wild-type non-human animal used as a control is made when evaluating the severity of the expression of Goodpasture's syndrome as an index.

6. (Amended) The method for screening a remedy for Goodpasture's syndrome according to claim 3, wherein at least one among diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane is found in the expression of Goodpasture's syndrome.

7. (Amended) The method for screening a remedy for Goodpasture's syndrome according to claim 3, wherein the non-human animal is a mouse.

Please add the following new claims.

--9. (New) The method for screening a remedy for Goodpasture's syndrome according to claim 4, wherein a comparative evaluation with a wild-type non-human animal used as a control is made when evaluating the severity of the expression of Goodpasture's syndrome as an index.

10. (New) The method for screening a remedy for Goodpasture's syndrome according to claim 4, wherein at least one among diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane is found in the expression of Goodpasture's syndrome.

11. (New) The method for screening a remedy for Goodpasture's syndrome according to claim 4, wherein the non-human animal is a mouse.--

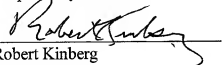
Applicant(s): Akira NAKAMURA et al.

REMARKS

This Preliminary Amendment is made to eliminate multiple claim dependency. Examination on the merits of the application is requested. A marked up version showing the changes made to the claims is attached.

Date: 12/14/01

Respectfully submitted,


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MARKED-UP VERSION OF CLAIMS

5. (Amended) The method for screening a remedy for Goodpasture's syndrome according to claim 3 [or 4], wherein a comparative evaluation with a wild-type non-human animal used as a control is made when evaluating the severity of the expression of Goodpasture's syndrome as an index.

6. (Amended) The method for screening a remedy for Goodpasture's syndrome according to [any one of claims 3 to 5] claim 3, wherein at least one among diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane is found in the expression of Goodpasture's syndrome.

7. (Amended) The method for screening a remedy for Goodpasture's syndrome according to [any one of claims 3 to 6] claim 3, wherein the non-human animal is a mouse.

3/PRTS

10/009950

JCO5 Rec'd POSTAGE 14 DEC 2007

SPECIFICATION

TITLE OF THE INVENTION

GOODPASTURE'S SYNDROME MODEL MOUSE

TECHNICAL FIELD

The present invention relates to a non-human model animal of Goodpasture's syndrome, a method for screening a remedy for Goodpasture's syndrome by using the model animal, and a method for diagnosing Goodpasture's syndrome at the early stage.

PRIOR ART

Goodpasture's syndrome, which has the combination of diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane antibody, is similar as a clinical feature with Wegener's granulomatosis, systemic necrotizing vasculitis, systemic lupus erythematosus (SLE) and the like, and is believed that antibodies common to the kidney glomerular basement membrane and alveolar epithelium basement membrane exist in the patient's serum, which bind to the target tissues and induce lesion of inflammation based on type II hypersensitivity reaction (J. Exp. Med. 126, 989-1004, 1987). Aside from the characteristics of the above-mentioned clinical feature, the diagnosis of Goodpasture's syndrome is made by proving the deposition of immunoglobulin (hereinafter "Ig") on the kidney glomerular basement membrane, where most of the anti basement membrane antibodies belong to the IgG fraction, and in recent years, it has been identified as an

autoantibody to a part of the α_1 chain of type IV collagen (Cell Mol. Biol. 5, 107-112, 1991).

Moreover, the onset of Goodpasture's syndrome is found in a wide range of age bracket, where without its diagnosis and therapy at the early stage, 80 % of the patients die within a year due to the deterioration of nephrosis, and 30 % of the patients die due to pulmonary hemorrhage. Recently, therapy at the early stage has enabled to increase the average of saving lives of Goodpasture's syndrome to about 50 %, but therapy only with the use of oral steroid agent or oral immunosuppressive drug is insufficient, and using high-dose of predonine pulse therapy is effective for pulmonary hemorrhage. However, pulse therapy is not sufficient for nephrosis, and is said that plasma exchange and high-dose of predonine, with the use of cyclophosphamide at the same time is effective, whereas to serious renal damage, artificial dialysis or renal transplantation is considered.

On the other hand, receptors that recognize and bind to the Fc portion of Ig (hereinafter "FcR") exist on the surface of cells in such as the immune system and the like, and the Fc γ receptor (hereinafter "Fc γ R") among them, which is a receptor protein that binds specifically to the γ chain of IgG in the body fluid, is classified mainly into three types, type I (CD64 antigen), type II (CD32 antigen), and type III (CD16 antigen), based on the similarity of gene structure. Among these, Fc γ RII differs from the other FcRs in that it has low affinity to the IgG of the monomer, binds to the polyvalent IgG that has become an immune complex, and is widely expressed in the hemopoietic stem cells including monocytes, macrophages, polymorphonuclear (PMN) leukocytes, mast cells, platelets,

some of the T cell lymphocytes and B cell lymphocytes. Moreover, three types of receptors having different gene arrangements, $Fc\gamma RIIA$, $Fc\gamma RIIB$, and $Fc\gamma RIIC$, exist in the $Fc\gamma RII$, and it is also known that each of the chromosomes are positioned in 1q23.

Unlike the other $FcRs$, the above-mentioned $Fc\gamma RIIB$ does not associate with γ chain, and has an amino acid sequence (ITIM: Immunoreceptor Tyrosine-based Inhibition Motif) which transmits suppressive signal to the intracellular domain (Immunol. Rev. 125, 49-76, 1992, Science 256, 1808-1812, 1992). In order to elucidate these physiological functions of $Fc\gamma RIIB$, the inventors of the present invention had already constructed $Fc\gamma RIIB$ -deficient mouse (Nature 379, 346-349, 1996), and constructed arthritis model mouse which is generated by immunizing $Fc\gamma RIIB$ -deficient mice with type II collagen (J. Exp. Med. 189, 187-194, 1999), and autoimmune disease model mouse (Japanese Laid-Open Patent Publication No.08-289699).

Model animals that are effective in the study on the pathogenesis of Goodpasture's syndrome, where 80 % of the patients die within a year due to the deterioration of nephrosis and 30 % among the patients die due to pulmonary hemorrhage without its diagnosis and therapy at the early stage, and in the development of therapy for Goodpasture's syndrome, had not been known to the present. An object of the present invention is to present a non-human model animal of Goodpasture's syndrome contributing to the treatment of Goodpasture's syndrome where the development of therapy had been delayed due to the lack of adequate disease models to elucidate its onset mechanism, a method for screening a remedy for Goodpasture's syndrome by using the model animal, and a method for diagnosing

Goodpasture's syndrome at the early stage.

SUMMARY OF THE INVENTION

The inventors of the present invention have conducted intensive study to elucidate the physiological functions of $\text{Fc}\gamma\text{RIIB}$, and have discovered that when a mouse whose function of $\text{Fc}\gamma\text{RIIB}$ gene is deficient on its chromosome, namely, the $\text{Fc}\gamma\text{RIIB}$ knockout mouse, is immunized with type IV collagen, said $\text{Fc}\gamma\text{RIIB}$ knockout mouse indicates diagnostic sign of Goodpasture's syndrome, and thus the present invention has been completed.

The present invention relates to a non-human model animal of Goodpasture's syndrome, especially Goodpasture's syndrome model mouse, which is obtained by immunizing a non-human animal whose function of immunoglobulin $\text{Fc}\gamma$ receptor IIB gene is deficient on its chromosome with type IV collagen or peptide which includes a part of its amino acid sequence.

Moreover, the present invention relates to a method for screening a remedy for Goodpasture's syndrome characterized in that: test substances are administered to a non-human animal whose function of immunoglobulin $\text{Fc}\gamma$ receptor IIB gene is deficient on its chromosome before, after or at the same time it is immunized with type IV collagen; and the severity of the expression of Goodpasture's syndrome as an index is evaluated, a method for screening a remedy for Goodpasture's syndrome characterized in that: test substances are administered to a non-human model animal of Goodpasture's syndrome; and the severity of the expression of Goodpasture's syndrome as an index is evaluated, the above-mentioned method for screening a remedy

for Goodpasture's syndrome characterized in that: a comparative evaluation with the wild-type non-human animal used as a control is made when the severity of the expression of Goodpasture's syndrome as an index is evaluated, the above-mentioned method for screening a remedy for Goodpasture's syndrome characterized in that: the expression of Goodpasture's syndrome is at least one among diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane antibody, and the above-mentioned method for screening a remedy for Goodpasture's syndrome characterized in that: the non-human animal is a mouse.

In addition, the present invention relates to a method for diagnosing Goodpasture's syndrome at the early stage, wherein a $\text{Fc}\gamma$ receptor IIB gene is extracted from human test cells, and is examined whether there is any deficiency in the function of said gene.

BRIEF EXPLANATION OF DRAWINGS

Fig. 1 is a view showing Goodpasture's syndrome-like alveolar hemorrhage by immunization with type IV collagen.

Fig. 2 is a view showing Goodpasture's syndrome-like glomerulonephritis by immunization with type IV collagen.

Fig. 3 is a graph showing the level of antibody titer of immunization with type IV collagen.

BEST MODE FOR CARRYING OUT THE INVENTION

In the present invention, a "non-human animal whose function of $\text{Fc}\gamma\text{RIIB}$ gene is deficient on its chromosome" means

a non-human animal whose function of expressing Fc γ RIIB is impaired by inactivation of endogenous genes of the non-human animal encoding Fc γ RIIB caused by genetic mutation such as destruction, deficiency, substitution or the like. Specific examples of "non-human animal" in the present invention include a rodent such as a mouse or a rat, but are not limited to these examples.

For example, mouse whose function of Fc γ RIIB gene is deficient on its chromosome, namely, the Fc γ RIIB knockout mouse, can be constructed by using the method as previously described (Nature 379, 346-349, 1996) by the present inventors, or the like. Specifically, a gene fragment obtained from a mouse genomic library using methods such as PCR or the like is used to screen the Fc γ RIIB gene, and the Fc γ RIIB gene which had been screened is subcloned by using a viral vector or the like, and is determined by DNA sequencing. A fragment that includes S₂ exon and EC₁ exon of the said clone was substituted with pMC1 neo gene cassette or the like, and a target vector was generated.

The linearized vector was introduced into ES cells by methods such as electroporation or the like, followed by homologous recombination, and ES cells indicating resistance to G418 and the like were selected from said homologous recombinants, and then the clone of said cells were microinjected into the blastocysts of the mice, said blastocysts were returned to the tentative parent mice, and chimeric mice were generated. These chimeric mice were intercrossed with wild-type mice to obtain heterozygous mice, and these heterozygous mice were intercrossed to obtain Fc γ RIIB knockout mice.

In the present invention, type IV collagen is a specific

exemplification of an immunogen used to induce Goodpasture's syndrome to a non-human animal whose function of Fc γ RIIB gene is deficient on its chromosome, however, anything such as a peptide which includes a part of the amino acid sequence of type IV collagen or the like can be used, as long as Goodpasture's syndrome can be induced to the non-human animal whose function of Fc γ RIIB gene is deficient on its chromosome.

In the present invention, a "non-human model animal of Goodpasture's syndrome" can be any kind of non-human animal, as long as it is a non-human animal such as a mouse or the like, which has the combination of three diagnostic signs of diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane antibody. For example, it can be obtained by immunizing a non-human animal whose function of Fc γ RIIB gene is deficient on its chromosome with type IV collagen.

Examples of methods for screening a remedy for Goodpasture's syndrome in the present invention are: a method in which test substances that are expectants for the remedy for Goodpasture's syndrome are administered to the non-human animal whose function of Fc γ RIIB gene is deficient on its chromosome before or after Goodpasture's syndrome is induced to the non-human animal by immunization with type IV collagen; or at the same time Goodpasture's syndrome is induced to the non-human animal by immunization with type IV collagen; and the severity of the expression of Goodpasture's syndrome (appearance of diagnostic signs) as an index is evaluated, and a method in which test substances that are expectants for the remedy for Goodpasture's syndrome are administered to the Goodpasture's syndrome non-human model animal; and the severity

of the expression of Goodpasture's syndrome as an index is evaluated.

In addition, when evaluating the severity of the expression of Goodpasture's syndrome as an index, a wild-type non-human animal of the same species as the non-human model animal of Goodpasture's syndrome can be used as a control, and a comparative evaluation of the severity of the expression of Goodpasture's syndrome between the non-human model animal of Goodpasture's syndrome and the wild-type non-human animal of the same species used as a control can be made.

As an index of the severity of expression (appearance of diagnostic signs) of Goodpasture's syndrome, at least one among diffuse alveolar hemorrhage in the pulmonary tissues, glomerulonephritis in the renal tissues, and the appearance of antikidney glomerular basement membrane antibody can be favorably exemplified, including other examples such as the expression of anti basement membrane antibody depositing in the pulmonary alveoli, serum creatinine level, the glomerular filtration value, or the like. Evaluating at least one of these indexes can screen the remedy for Goodpasture's syndrome.

In the present invention, a specific method for diagnosing Goodpasture's syndrome at the early stage is to extract Fc γ RIIB gene from human test cells, and examine whether there is any deficiency in the function of said gene. Examples of human test cells used as Fc γ RIIB gene source are macrophages, mast cells, B cells, dendritic cells and the like, and an example of a method for examining whether there is any deficiency in the function of Fc γ RIIB gene is to express the cloned Fc γ RIIB gene in the human cell line by ordinary method, and examine the function of Fc γ RIIB of the expression product, such as the

binding to IgG immune complex. Onset of Goodpasture's syndrome is possible when there is a deficiency in the function of Fc γ RIIB in the expression product, and as mentioned above, a method for diagnosing Goodpasture's syndrome at the early stage becomes possible by examining whether there is any deficiency in the function of Fc γ RIIB gene.

The present invention will now be explained more specifically with the following examples, however, the technical scope of the invention is not limited to these examples.

Reference (Generation of Fc γ RIIB-deficient mice)

A genomic DNA clone for Fc γ RIIB gene was isolated by screening a 129/Sv/J-lineage-derived mouse genomic DNA library. A targeting vector was constructed by replacing a 2.65 Kb fragment which includes two separate exons of S₂ and EC₁ of said clone to a pMC1 neo gene cassette (Toyobo Co., Ltd.). This linearized vector was introduced into ES cells (J1) by electroporation, and was homologously recombined.

The ES clone was isolated from the ES cells that were homologously recombined as mentioned above, a neomycin-resistant ES clone was screened to G418 and GANC (ganciclovir), and homologous recombinants were identified by Southern blot. Genomic DNA isolated from the identified homologous recombinants was digested with Hind III, and the existence of targeting allele containing pMC1 neo gene cassette was confirmed. The said identified ES clone was microinjected into the blastocysts to generate chimeric mice, and the generated mice were intercrossed with wild-type C57BL/6J mice to obtain heterozygous mice, then these heterozygous mice were

intercrossed to obtain homozygous mice, and mice whose Fc γ RIIB gene is deficient on its chromosome and its wild-type mice were generated.

Example 1. (Construction of Goodpasature's syndrome model mice)

A Cellmatrix IV (Nitta Gellatin, Inc.) prepared from bovine crystalline lens at 3 mg/ml protein concentration in 1 mM HCL solution (pH 3.0), was added NaOH to a final concentration of 1 mM, to generate type IV collagen (pH 8.0). Two types of emulsions were generated by mixing 3mg/ml of said type IV collagen (pH 8.0) and 3 mg/ml of complete Freund's adjuvant (CFA) comprised of liquid paraffin, surface-active agent, and dead Mycobacterium tuberculosis in a connected syringe, and by mixing 3mg/ml type IV collagen (pH 8.0) and 3mg/ml incomplete Freund's adjuvant (IFA) comprised of liquid paraffin and surface-active agent in a connected syringe.

The Fc γ RIIB gene deficient mice (eight weeks of age: gender at randomly chosen) generated from the method described in the above-mentioned reference were anesthetized by ether and its tail base were shaved, and 100 μ l emulsion containing 150 μ g each of type IV collagen and CFA were injected to the mice's skin for primary immunization. After the primary immunization, on day 14, day 28, and day 42, 100 μ l emulsion containing 150 μ g each of type IV collagen and IFA were injected to their skin, the mice were killed on day 56, and the pulmonary tissues and renal tissues were extracted. In addition, wild-type mice were used as a control.

As shown in Fig.1, Fc γ RIIB gene deficient mice immunized with type IV collagen (Fc γ RIIB^{-/-}), compared to control wild-type mice (WT), showed remarkable signs of alveolar

hemorrhage in the pulmonary tissues at a wide range including the infiltration of inflammatory cells such as macrophages, neutrophils, and the like. In addition, as shown in Fig.2, degeneration of glomus and proximal renal tubule in the renal tissues were displayed and renal lesion mainly as glomerulonephritis occurred. From these results, it can be understood that Goodpasture's syndrome model mice can be obtained by immunizing Fc γ RIIB gene deficient mice with type IV collagen.

Example 2 (Examination of antibody titer to type IV collagen)

After each of Fc γ RIIB knockout mice, FcR γ knockout mice, and wild-type mice were immunized with type IV collagen, blood was extracted from the orbit after a set period of time, and the antibody titer to type IV collagen was tested by the following method, where improvement had been added to the ELISA analysis previously described (Cell. Immunol. 145, 299-310, 1992).

20 μ g type IV collagen was lysed in 1 ml phosphate buffered solution (PBS), and this lysate solution was used at 50 μ l/well, and after coating a 96-well microplate (Falcon; Becton Dickinson Labware) at 4 C° for overnight, was washed three times with PBS containing 0.05% Tween 20 and 0.1% BSA, and then blocked with PBS containing 0.2% BSA at 250 μ l/well at 4 C° overnight.

The serum obtained from the blood mentioned above was then diluted to 400 to 20000 times, and the diluted serum was added to the aforementioned 96-well microplate at 50 μ l/well, and allowed to react at 4 C° overnight. After the reaction, the 96-well microplate was washed three times with PBS containing 0.05 % Tween 20, added 50 μ l of horseradish peroxidase (Sigma

Chemical Co.)-conjugated goat anti-mouse IgG1, IgG2a, or IgG2b diluted to 200 times, and was then incubated at 4 C° for 2 hours. After incubation, it was washed again three times with PBS containing 0.05% Tween 20, and developed enzyme reaction at room temperature for 30 minutes with 0.1 ml of True Blue Peroxidase Substrate (Kirkegaard & Perry Labs). The OD 450 was then read by using a Microplate Reader (Biolumin960; Molecular Dynamics Japan, Inc.). The results are shown in Fig. 3.

From these results, increase in antibody titer to type IV collagen (IgG1, IgG2a, IgG2b or IgM) can be seen in Fc γ RIIB knockout mice (IIB-KO), compared to FcR γ knockout mice (γ -KO) and wild-type mice (Wild), and since this is not inconsistent with the observations to Goodpasture's syndrome, it was found that Goodpasture's syndrome model mouse was generated.

INDUSTRIAL APPLICABILITY

According to the present invention, a non-human model animal of Goodpasture's syndrome, a method for screening a remedy for Goodpasture's syndrome using the model animal, and a method for diagnosing Goodpasture's syndrome at the early age can be provided, which leads to the therapy of Goodpasture's syndrome, where the development of therapy had been delayed due to the lack of adequate disease models for elucidating its onset mechanism.

WHAT IS CLAIMED IS

1. A non-human model animal of Goodpasture's syndrome characterized in that a non-human animal whose function of immunoglobulin Fc γ receptor IIB gene is deficient on its chromosome is obtained by immunizing with type IV collagen.

2. The non-human model animal of Goodpasture's syndrome according to claim 1, wherein the non-human animal is a mouse.

3. A method for screening a remedy for Goodpasture's syndrome characterized in that test substances are administered to a non-human animal whose function of immunoglobulin Fc γ receptor IIB gene is deficient on its chromosome before or after immunizing or at the same time the said non-human animal is immunized with type IV collagen, and the severity of the expression of Goodpasture's syndrome as an index is evaluated.

4. A method for screening a remedy for Goodpasture's syndrome characterized in that test substances are administered to the non-human model animal of Goodpasture's syndrome and the severity of the expression of Goodpasture's syndrome as an index is evaluated.

5. The method for screening a remedy for Goodpasture's syndrome according to claim 3 or 4, wherein a comparative evaluation with a wild-type non-human animal used as a control is made when evaluating the severity of the expression of Goodpasture's syndrome as an index.

6. The method for screening a remedy for Goodpasture's syndrome according to any one of claims 3 to 5, wherein at least one among diffuse alveolar hemorrhage, glomerulonephritis, and the appearance of antikidney glomerular basement membrane is found in the expression of Goodpasture's syndrome.

7. The method for screening a remedy for Goodpasture's syndrome according to any one of claims 3 to 6, wherein the non-human animal is a mouse.

8. A method for diagnosing Goodpasture's syndrome at the early stage characterized in that a Fc γ receptor IIB gene is extracted from human test cells and is examined whether there is any deficiency in the function of said gene.

Fig.1

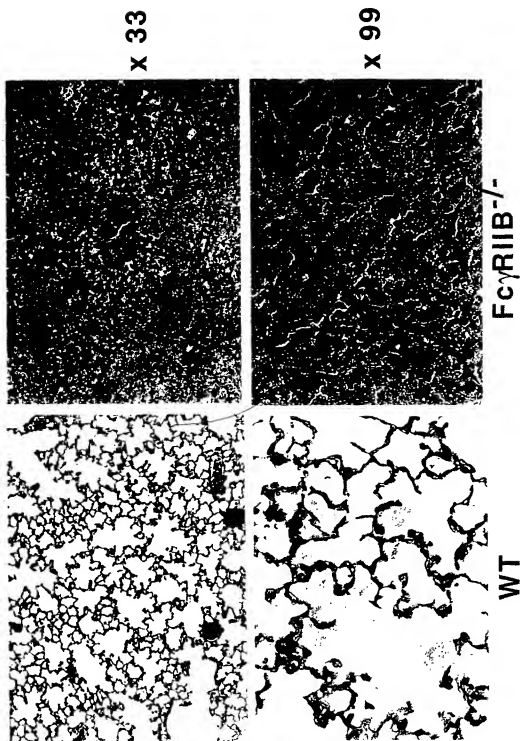
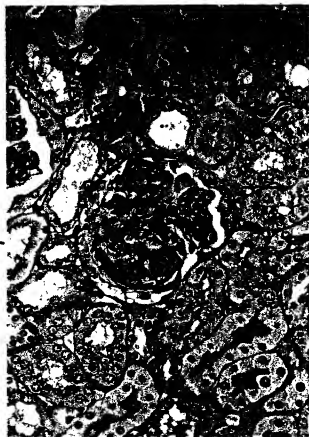


Fig. 2

FC γ RIIB^{-/-}



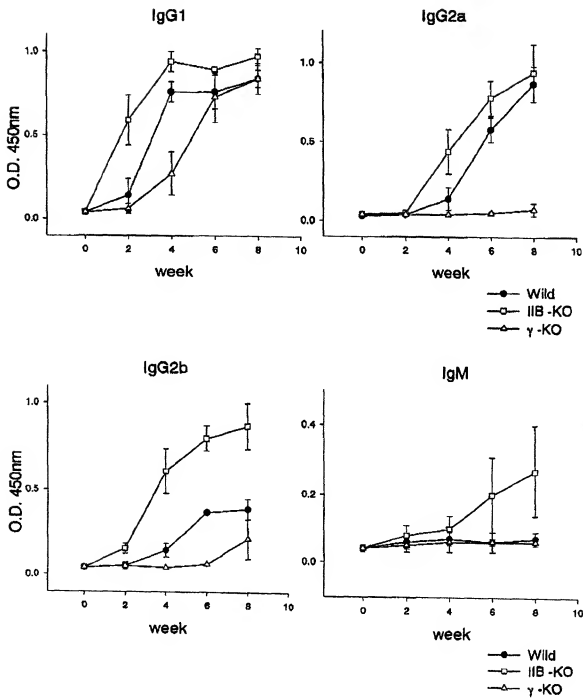
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x 100

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Fig.3



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Attorney Docket
31671-176197 RK

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As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled GOODPASTURE'S SYNDROME MODEL MOUSE, the specification of which

[] is attached hereto.

[] was filed on _____, as Application Serial No. _____, Confirmation No. _____, and was amended on _____ [if applicable].

[X] was filed under the Patent Cooperation Treaty on June 23, 2000 Serial No. PCT/JP00/04132, the United States of America being designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, 119 of any foreign application(s) for patent, utility model, design or inventor's certificate listed below and have also identified below any foreign application(s) for patent, utility model, design or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
Number	Country	Date Filed	Yes	No
H11-180600	JAPAN	June 25, 1999	X	

I hereby appoint the attorneys and agents of VENABLE associated with the following customer number to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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The undersigned hereby authorizes the U.S. attorneys identified herein to accept and follow instructions from the undersigned's assignee, if any, and/or, if the undersigned is not a resident of the United States, the undersigned's domestic attorney, patent attorney or patent agent, as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and the undersigned. In the event of a change in the person(s) from whom instructions may be taken, the U.S. attorneys identified herein will be so notified by the undersigned.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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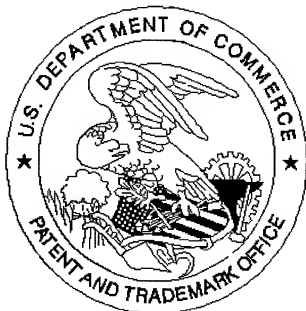
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